

requirement. Claims 17, 19 and 20 have been amended to correct obvious inadvertent clerical and typographical errors, and claims 16 and 18 have been amended to better claim the subject matter which Applicants regard as their invention. The amendments to the Specification have been made to correct obvious, inadvertent typographical errors. None of the amendments made herein constitutes the addition of new matter.

The Formal Drawings

Formal drawings were received by the Patent Office on September 18, 2001, per the date stamped on the return receipt postcard sent by to Applicants. Applicants respectfully request written confirmation of acceptance of the drawings.

The Requirement for Restriction

The Patent Office has required restriction under 35 U.S.C. 121, alleging that the claims embody three patentably distinct inventions:

- I. Claims 1-6, drawn to DNA and expression vectors encoding truncated ultraviolet damage endonuclease (UVDE)
- II. Claims 7-15, drawn to truncated UVDE and compositions comprising it.
- III. Claims 16-20, drawn to method of cleavage of a double-stranded DNA with truncated UVDE

1. 2. 3. 4. 5. 6. 7. 8. 9. 10. 11. 12. 13. 14. 15. 16. 17. 18. 19. 20.
Applicants confirm the telephonic election (with traverse) of the claims of Group III for examination. Claims 1-15 have been canceled without prejudice in the present Amendment.

The Rejections under 35 U.S.C. 112, second paragraph

Claims 17 and 19 have been rejected under 35 U.S.C. 112, second paragraph, as allegedly lacking antecedent basis. The Examiner has correctly concluded that the recitation of "method" rather than composition was intended.

Applicants have amended the claims to recite "method" for consistency with the base claims.

/The Rejections under 35 U.S.C. 102

Claims 16, 18 and 20 have been rejected under 35 U.S.C. 102(b) as allegedly anticipated by Takao et al. (1996), Yajima et al. (1995), Bellacosa et al. (1999) and Hendrich et al. (1998). Applicants respectfully traverse this rejection.

The Takao reference

The Patent Office has alleged that SEQ ID NO:2, amino acids 230-828, is taught at Fig. 2 (pg. 1269) and the incision assay for double-stranded DNA is described at page 1268.

A careful reading of the Takao reference reveals that this reference teaches that the truncated endonuclease was not stable in pure form, and that the assays were carried out with endonuclease preparations which were only about 35% pure. See page 1269, column 1. By contrast, the present application teaches that the truncated UVDE proteins were purified to apparent electrophoretic homogeneity and that the proteins made were stable in pure form. Claims 16 and 18 has been amended to specify that the endonuclease based on SEQ ID NO:2, amino acids 230-828, is purified. Thus, Applicants respectfully submit that the present claimed invention is distinguished over the teachings of the cited Takao reference.

In view of the above discussion of the reference and the amendment to the claims, Applicants respectfully submit that the invention is not anticipated by the cited Takao reference, and the withdrawal of this aspect of the rejection is requested.

The Yajima reference

The Patent Office has alleged that SEQ ID NO:36 is shown in Fig. 2 (pg. 2394) and the incision assay is taught at page 2399.

The cited Yajima reference relates to UV damage specific endonuclease from *Neurospora crassa*. The first incision assay taught at page 2399 is one in which closed circular plasmid DNA has been UV-irradiated. The second assay is one in which oligonucleotides with pyrimidine dimers have been UV-irradiated.

Without acquiescing to this rejection and in the interest of advancing prosecution, Applicants have amended claims 16 and 18 to specify that when the endonuclease has the amino acid sequence given in SEQ ID NO:36, and that the distortion is due not to a photoproduct in an oligonucleotide or in a closed circular plasmid DNA.

In view of the amendments to the claims, Applicants respectfully urge that the cited Yajima reference does not anticipate the invention as presently claimed, and the withdrawal of the rejection is requested.

The Bellacosa reference

The Patent Office has alleged that SEQ ID NO:38 is presented in Fig. 2 (pg. 3972) and the method for DNA cleavage is taught at page 3970.

Applicants respectfully maintain that the method for DNA cleavage taught at page 3970 of the cited Bellacosa reference is a method for cleavage of supercoiled plasmid DNA. There is no indication in this cited reference that the DNA contains any distortion(s) corresponding to those particularly recited in the instant claims. In the absence of any specific notation of mismatch, photoproducts, loop, etc., one of ordinary skill in the art will assume that there is no distortion in the supercoiled DNA.

In view of the foregoing clarification of the teaching of the endonuclease assay in the cited Bellacosa reference, Applicants respectfully maintain that the cited reference does not anticipate the invention as claimed and this aspect of the rejection must be withdrawn.

The Hendrich reference

The Patent Office has not specifically discussed the Hendrich reference, and Applicants have reviewed this reference and make the following comments. All of the data presented in the cited Hendrich reference appear to be directed to sequence comparisons (Figures 1 and 2) and to the binding of certain proteins to methylated DNAs. The Patent Office is requested to point out the passage of this reference which relates to endonucleolytic cleavage of damaged DNA.

The undersigned has obtained and reviewed a copy of the NCIB database entry AF072250, which contains the nucleotide and deduced amino acid sequence of the fourth protein discussed in the cited Hendrich reference. The GenBank report does not identify this protein as an endonuclease but rather as the methyl-CpG binding protein MBD4. A copy is attached for the Examiner's convenience.

Applicants further note that the Hendrich reference was published after the filing date of June 8, 1999 of USSN 09/327,984, from which priority is claimed. That application has issued as US Patent No. 6,368,594 B1. SEQ ID NO:38 appears in that application/patent.

In view of the apparent failure of the Hendrich reference to disclose a reaction in which the MBD4 protein exhibits distortion-specific endonuclease activity, and in view of the disclosure of SEQ ID NO:38 in the parent application, Applicants respectfully maintain that the reference does not anticipate the present claimed invention, and the withdrawal of this aspect of the rejection is requested.

Conclusion

In view of the foregoing, it is submitted that this case is in condition for allowance, and passage to issuance is respectfully requested.

If there are any outstanding issues related to patentability, the courtesy of a telephone interview is requested, and the Examiner is invited to call to arrange a mutually convenient time.

This Amendment is accompanied by a Petition for Extension of Time (two months) and a check in the amount of \$200.00 as required by 37 C.F.R 1.17. It is believed that this amendment does not necessitate the payment of any additional fees under 37 C.F.R. 1.16-1.17. If this is incorrect, however, please charge any additional fees due under 37 C.F.R. 1.16-1.17 to Deposit Account No. 07-1969.

Respectfully submitted,



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Marked up version of amended paragraph(s) and claim(s) in attached Amendment.

Docket No.: 25-98A
USSN 09/724,296

The last paragraph on page 4:

It is an object of the present invention to provide purified stable UVDE (Uve1p), polypeptide fragments which retain high levels of activity, particularly those from the *Schizosaccharomyces pombe* enzyme. In a specific embodiment, the polypeptide fragment is Δ 228-UVDE, which contains a 288-228 amino-acid deletion of the N-terminal region of the *S. pombe* *uve1* + gene product; a second specific embodiment is the fusion protein ~~GST-~~ Δ 228-UVDE.~~GST-~~ Δ 228-UVDE. The DNA sequence encoding GST-full-length UVDE from *S. pombe* is given in SEQ ID NO:1. The deduced amino acid sequence of full-length UVDE is given in SEQ ID NO:2. The DNA sequence encoding Δ 228-UVDE is given in SEQ ID NO:3. The deduced amino acid sequence of Δ 228-UVDE is given in SEQ ID NO:4. The DNA coding sequence and deduced amino acid sequence for GST- Δ 228-UVDE are given in SEQ ID NO:5 and SEQ ID NO: 6, respectively. Also encompassed within the present invention are truncated UVDE proteins wherein the truncation is from about position 100 to about position 250 with reference to SEQ ID NO:2, and wherein the truncated proteins are stable in substantially pure form.

The second full paragraph on page 6:

Another embodiment of the invention features a hybridoma which produces an antibody having specific binding affinity to a UVDE polypeptide fragment. By "hybridoma" is meant an immortalized cell line which is capable of secreting an antibody, for example a Δ 228-UVDE specific antibody. In preferred embodiments, the UVDE specific antibody comprises a sequence of amino acids that is able to specifically bind ~~Δ 288-UVDE~~. Δ 228-UVDE. Alternatively, a GST-tag specific

antibody or labeled ligand could be used to determine the presence of or quantitate a GST- Δ 228-UVDE polypeptide, especially in formulations *ex vivo*.

The paragraph bridging pages 11-12:

Fig. 10A shows cleavage of an oligonucleotide substrate containing an AP site by Uve1p. To investigate if Uve1p was capable of cleaving an abasic site in a hydrolytic manner, we prepared a 5' end-labeled (*) abasic substrate, AP-37mer, and incubated this substrate with buffer alone (lane 1), *E. coli* endonuclease III (AP lyase, lane 2), affinity-purified G Δ 228-Uve1p and Δ 288-Uve1p, Δ 228-Uve1p (2 μ g of each) (lanes 3 and 4), extracts of cells over-expressing G Δ 288-Uve1p, G Δ 228-Uve1p (5 μ g) (lane 5), *E. coli* endonuclease IV (hydrolytic AP endonuclease, lane 6) and purified recombinant GST (2 μ g) (lane 7). Fig. 10B demonstrates competitive inhibition of AP site recognition and cleavage. To demonstrate that the products generated are as a result of Uve1p-mediated cleavage at the AP site, AP-37mer was incubated with buffer alone (lane 1), *E. coli* endonuclease IV (lane 2), and affinity-purified G Δ 228-Uve1p (2 μ g) (lane 3) with 10X and 40X unlabeled cs-CPD-30mer (lanes 4 and 5, respectively) and 10X and 40X unlabeled UD-37mer (lanes 6 and 7, respectively). Arrows **a** and **b** indicate the primary and secondary Uve1p-mediated cleavage products, respectively. Arrow **uc** indicates the uncleaved substrate. A portion of the sequence of the AP substrate is shown at the bottom of the figure. S corresponds to deoxyribose and p corresponds to phosphate. The location of the cleavage sites of endonuclease III (E_{III}) and endonuclease IV (E_{IV}) are also indicated. For simplicity the complementary strand is omitted from the figure.

The paragraph bridging pages 22-23:

Uve1p isolated from *S. pombe* was first described as catalyzing a single ATP-independent incision event immediately 5' to the UV photoproduct, and generating termini containing 3' hydroxyl and 5' phosphoryl groups (Bowman et al. [1994] *Nucl. Acids Res.* **22**:3026-3032). The purified G Δ 288-Uve1p, Δ 288-Uve1p, G Δ 228-Uve1p, Δ 228-Uve1p and crude cell lysates of recombinant G-

Uve1p and GΔ228-Uve1p GΔ228-Uve1p make an incision directly 5' to CPDs similar to that observed with the native protein. In this study, we have used both 5' and 3' end-labeled duplex CPD-30mer (cs-CPD-30mer) to demonstrate the ability of Uve1p to cleave a CPD-containing substrate at two sites (Fig. 6A-6B). The primary product (arrow a) accounted for approximately 90% of the total product formed and resulted from cleavage immediately 5' to the damage. The second incision site was located one nucleotide upstream and yielded a cleavage product (arrow b), which represented the remaining 10% of the product formed. This minor product is one nucleotide shorter or longer than the primary product depending on whether 5' or 3' end-labeled substrate is being examined. The same cleavage pattern was observed for each different Uve1p preparation used: i.e., crude extracts of cells expressing GΔ228-Uve1p, affinity-purified GΔ228-Uve1p and Δ228-Uve1p (Fig. 2A and 2B, lanes 2, 3 and 4 respectively), as well as extracts of cells expressing GST-Uve1p. No cleavage products were observed when the cs-CPD-30mer substrates were incubated with buffer only, or purified recombinant GST prepared and affinity-purified in an identical manner to the purified Uve1p proteins (Fig. 6A, 6B, lanes 1 and 5 respectively). This control eliminates the possibility that these DNA strand scission products are formed as a result of the presence of trace amounts of non-specific endonuclease contamination. Uve1p recognizes a duplex cs-CPD-containing oligonucleotide substrate and cleaves this substrate at two sites. The primary site, responsible for 90% of the product, is immediately 5' to the damage and the secondary site (accounting for the remaining 10% of product), is one nucleotide 5' to the site of damage.

The full paragraph at page 29, lines 6-25:

Uve1p has been shown to incise DNA containing CPDs and 6-4PPs directly 5' to the photoproduct site generating products containing 3'-hydroxyl and 5'-phosphoryl groups [Bowman et al. (1994) *supra*]. We examined whether similar 3' and 5' termini were produced following Uve1p-mediated cleavage of base mismatch-containing substrates. DNA strand scission products generated by GΔ228-Uve1p cleavage of 3' end-labeled oligo *CX/AY-31mer (CX strand labeled, Table 1B) were further treated with calf intestinal phosphatase (CIP) which removes 5' terminal phosphoryl groups from substrate DNA. The major sites of Uve1p-mediated DNA cleavage relative

to the base mispair site were found to be at positions 0 and -1 (Fig. 11A, lane 2). CIP treatment of these DNA cleavage products resulted in species that had retarded electrophoretic mobilities compared to non-CIP-treated DNA cleavage products, indicating a decrease in charge corresponding to removal of 5' terminal phosphoryl groups (Fig. 11A, lanes 2 and 3). In addition, GΔ228-Uve1p mismatch endonuclease-generated DNA cleavage products were resistant to phosphorylation by polynucleotide kinase, an expected result if the 5' termini already contain phosphoryl groups (Fig. 11A, lane 4). Electrophoretic ~~mobility~~ shift analysis utilizing 5' end-labeled *CX/AY-31mer, terminal deoxyribonucleotidyl transferase (TdT), and $\alpha^{32}\text{P}$ -dideoxyATP (ddATP) resulted in addition of a single ddAMP to the 3' end of GΔ228-Uve1p-generated DNA cleavage products and indicates the presence of a 3'-hydroxyl terminus. These results show that the 3' and 5' termini of the products of GΔ228-Uve1p-mediated cleavage of substrates containing single base mismatches are identical to those generated following cleavage of substrates containing

The title of Table 13 at page 70:

Table 13. Neospora Neurospora crassa UVDE Homolog (Genbank Accession No. BAA-74539)
SEQ ID NO:36)

In the claims:

16. (Once amended) A method for cleavage of a double-stranded DNA molecule characterized by a distorted structure, wherein said distorted structure results from ultraviolet radiation damage, a photoproduct, an abasic site, mismatched nucleotide pairing, a platinum diadduct, an intercalated molecule, an insertion deletion loop of five or fewer nucleotides or alkylation of a nucleotide or a uracil residue resulting from deamination of a cytosine residue, said method comprising the step of contacting a DNA molecule characterized by a distorted structure with a broadly specific DNA damage endonuclease selected from the group of endonucleases selected from the group consisting of an endonuclease identified by the amino acid sequence [as] given in SEQ ID NO:2 wherein said endonuclease is

purified, amino acids 230 to 828; a truncated stable truncated Uve1p identified by the amino acid sequence given in SEQ ID NO:4; the endonuclease identified by the amino acid sequence given in SEQ ID NO:36; the endonuclease identified by the amino acid sequence given in SEQ ID NO:37; the endonuclease identified by the amino acid sequence given in SEQ ID NO:38; the endonuclease identified by the amino acid sequence given in SEQ ID NO:39, under conditions allowing for enzymatic activity of said endonuclease and wherein the double stranded DNA molecule is not an oligonucleotide which has been irradiated with ultraviolet light or a closed circle plasmid DNA molecule which has been irradiated with ultraviolet light when the endonuclease is identified by the amino acid sequence of SEQ ID NO:36.

17. (Once amended) The [composition] method of claim 16 wherein said truncated Uve1p has an amino acid sequence as given in SEQ ID NO:4.
18. (Once amended) A method for cleavage of a double-stranded DNA molecule characterized by a distorted structure, wherein said distorted structure results from ultraviolet radiation damage, a photoproduct, an abasic site, mismatched nucleotide pairing, a platinum diadduct, an insertion deletion loop, alkylation of a nucleotide, the presence of a uracil residue resulting from deamination of a cytosine residue, said method comprising the step of contacting a DNA molecule characterized by a distorted structure with a broadly specific DNA damage endonuclease selected from the group of endonucleases selected from the group consisting of an endonuclease [identified by] consisting of the amino acid sequence [as] given in SEQ ID NO:2, amino acids 230 to 828 wherein said endonuclease is purified; an endonuclease consisting of the sequence given in SEQ ID NO:6, a truncated stable truncated Uve1p identified by the amino acid sequence given in SEQ ID NO:4; the endonuclease identified by the amino acid sequence given in SEQ ID NO:36; the endonuclease identified by the amino acid sequence given in SEQ ID NO:37; the endonuclease identified by the amino acid sequence given in SEQ ID NO:38; the

endonuclease identified by the amino acid sequence given in SEQ ID NO:39, under conditions allowing for enzymatic activity of said endonuclease.

19. (Once amended) The [composition] method of claim 18 wherein said truncated Uve1p has an amino acid sequence as given in SEQ ID NO:4.
20. (Once amended) The method of claim 16 wherein the [ninsertion] insertion deletion loop is of four or fewer nucleotides.